Genetic interaction between two VNTRs in the MAOA gene is associated with the nicotine dependence

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Running Title

MAOA and nicotine dependence in Vietnam

Abstract

Nicotine dependence is an addiction to tobacco products and a global public health concern that in part would be influenced by our genetics. Smokers are reported to have reduced MAOA activity, but the results from genetic associations with this gene have been inconclusive. Two functionally relevant variable number tandem repeat (VNTR) domains, termed uVNTR and dVNTR, in the MAOA gene are well characterised transcriptional regulatory elements. In the present study we analysed uVNTR and dVNTR polymorphisms in the MAOA gene in the Vietnamese male population of smokers and non-smokers in order to assess the association of MAOA with the nicotine dependence measured by the Fagerström Test for Nicotine Dependence (FTND). Individual analysis of VNTRs separately identified uVNTR to be associated with the F6 question of the FTND indicating the stronger addiction to nicotine. No associations were found between the dVNTR and smoking behaviour. The combination of dVNTR and uVNTR, that predicts low expression of MAOA (10-3 haplotypes), was significantly associated with the higher nicotine dependence (FTND score), longer smoking duration and more persistent smoking behaviour (fewer quit attempts). In conclusion, our study confirms that low MAOA expression is genetically predictive to the higher nicotine dependence.

Keywords: Variable Number Tandem Repeat (VNTR), MAOA, smoking, nicotine dependence, Fagerström Test for Nicotine Dependence (FTND), Vietnam, genetic interaction, stratification

Impact statement

The present study combined analysis of two transcriptional regulators, uVNTR and dVNTR, in the MAOA gene that is an enzyme responsible for the monoamine degradation and identified genetic interaction between these VNTRs in association with the nicotine dependence. The main impact is that when analysing different populations in the genetic studies, the functionally meaningful variants should be combined rather than addressing individual elements separately (a mini polygenic risk score for a particular gene/locus). This combination is very rarely analysed and therefore the study sets an example. Another impact is that we analysed the genetic variability in the Asian population and therefore our data present a piece of information from underrepresented populations.

Introduction

Monoamine oxidase A (MAOA) is a member of the family of enzymes catalysing the oxidative deamination of amines, such as dopamine, norepinephrine and serotonin [1]. Humans have two enzymes, MAOA and MAOB, that are encoded by the tail-to-tail arranged genes in the X chromosome [2, 3]. Both enzymes localise in the mitochondrial outer membrane. While both, MAOA and MAOAB inactivate monoamine neurotransmitters, MAOA preferentially degrades serotonin, dopamine, melatonin, norepinephrine and epinephrine [4]. This preference explains why MAOA has been extensively studied in the context of behavioural traits and psychiatric diseases [5].

The MAOA gene is transcribed in two main protein coding variants, major and minor isoforms [6]. In its promoter and 5'UTR region, the MAOA gene has two variable number tandem repeats (VNTRs) that regulate differential expression of these two isoforms [6]. The proximal VNTR, uVNTR, is located around 1.2 kb upstream of one of the transcriptional start sites and it consists of a repeated 30-bp motif that can be present in 2, 3, 3.5, 4 and 5 copies [7]. 3.5 and 4 copy versions of uVNTR are more efficient as positive regulators and MAOA is expressed up to 10-times higher than with other uVNTR variants [7]. Recently, another VNTR, designated as a dVNTR, was identified 1500 bp upstream of the ATG site [8]. This variant is a 10-bp motif that can exist in 8, 9, 10 or 11 copies and these variants exhibit differential transcriptional activity [8]. In a cell line model the 9-copy variant is the most active, 8 and 11 have intermediate activity and 10 repeat version has the lowest transcriptional activity [8]. The authors of this initial study showed that dVNTR could have a stronger regulatory function than uVNTR over MAOA expression. Indeed, this was further confirmed in an independent study where the isoform specific effects of dVNTR and uVNTR repeats were described [6].

Differences in the MAOA activity or expression are related to impulsivity, behavioural disturbances and addictive disorders [9, 10] . MAOA activity in part is determined by the VNTR genotypes and persons with the low activity alleles can respond with violent and antisocial behaviour to environmental stress [11] . Substance abuse could be a result of the anti-social or impulsive behaviour induced by the stress. The most prevalent substance abuse is tobacco smoking that is the single major cause for premature death [12]. Nicotine

dependence is a dominant driving force that prevents successful quitting and causes a persistence of the smoking. Genetic architecture and its interaction to the environmental modifiers play a pivotal role in the coping reaction to challenging situations [13] . Variations in the uVNTR of the MAOA have been shown to be part of this interaction [14, 15]. However, most of the studies have focused on the associations with solely uVNTR and smoking and no study has been done to analyse the effect of dVNTR on the nicotine dependence. Taken into account the published evidence that uVNTR and dVNTR regulate the activity of the MAOA, the goal of present study was to evaluate the effects of uVNTR and dVNTR variants upon the nicotine dependence and to analyse the genetic interactions between these two tandem repeats. These VNTR interact to regulate the expression of MAOA gene therefore, it is plausible to assume that this interaction is also evident in the association study as we found in earlier study with 5HTT[16]. Nicotine dependence was measured using Fagerström Test for Nicotine Dependence (FTND) test that has become a standard test for similar type of studies. Association analysis between the variants of dVNTR or uVNTR and FTND total score or individual questions was performed to identify the impact of MAOA variants on smoking behaviour.

Methods

Study design and participants

The details of the study cohort have been described in our previous publications [16, 17]. Briefly, it is a community based cross-sectional study based on the nicotine dependence survey conducted in Da Nang and Hue, Vietnam. The cohort consists of 1,822 participants (1,453 smokers and 369 non-smokers). Only male subjects were included in the study as smoking is not prevalent in women in Vietnam. The Ethics Review Committees on Human Research of the University of Tartu, Da Nang University of Medical Technology and Pharmacy and Hue University of Medicine and Pharmacy approved the protocols and informed consent forms used in this study. All participants signed a written informed consent form during the completion of the questionnaire.

Smoking data

Smokers were defined as individuals who had smoked at least for one year. Threshold of one year was applied to enable sufficient time for development of nicotine dependence in a subject and this excludes the persons who only tried occasionally and never became regular smokers. The collected data was then inserted into the Red-Cap based database (https://www.project-redcap.org) [18]. RedCap is an abbreviation for the Research Electronic Data CAPture and is an innovative software to support clinical and translational research. RedCap is an ideal platform for similar study designs as it allows rapid development and deployment of electronic data capture which can be used in a clinical setting. The details of the questionnaire are published elsewhere [16]. Briefly we collected the information according to the FTND questionnaire with some additional general questions characterising the smoking behaviour.

Saliva collection and DNA extraction

For DNA collection and extraction Norgen saliva collection, preservation and isolation kit (RU35700, https://norgenbiotek.com) was used. For DNA collection, 2 ml of saliva was mixed with the preservative reagent and DNA was extracted according to the manufacturers protocol using the method described elsewhere [16] .

DNA genotyping

Assays for genotyping the uVNTR and dVNTR are described in the publication of Manca et al. [6] Two separate polymerase chain reactions (PCR) were used along with two different primer pairs (Table 1) to amplify uVNTR (291 bp, 321 bp, 352 bop and 381 bp) and dVNTR (345 bp, 355 bp, 365 bp and 375 bp) fragments. For the uVNTR the final reaction volume (20 ul) contained 1x AmpliTaq Gold MasterMix (Applied Biosystems, California, CA) and oligonucleotide primers (TAG Copenhagen A/S) (Table 1) at final concentration of 5 pmol with the 10 ng of gDNA. Thermal cycling consisted of 2 min of initial denaturation at 95^{o}C followed by 35 cycles of 95^{o}C (20s), 61^{o}C (20s) and 72^{o}C (30s) with a final extension step of 5 min at 72^{o}C. Subsequently, 10ul of PCR product was loaded onto a 1.5% agarose gel, run for 1h and 10 min at 160V in TBE and visualized by ethidium bromide.

For the dVNTR the final reaction volume (20 ul) contained 1x Hot FirePol GC Master Mix (Solis Biodyne, Tartu, Estonia), nucleotide mix with deaza-dGTP and oligonucleotide primers (TAG Copenhagen A/S) (Table 1) at final concentration of 5 pmol with the 10 ng of gDNA. Thermal cycling consisted of 2 min of initial denaturation at 95^{o}C followed by 10 cycles of 95^{o}C (20s), 65-55^{o}C (20s) and 72^{o}C (30s), then by 35 cycles of 95^{o}C (20s), 55^{o}C (20s) and 72^{o}C (30s) with a final extension step of 1 min at 72^{o}C. Subsequently, 10ul of PCR product was loaded onto a 2% agarose gel, run for 1h and 10 min at 160V in TBE and visualized by ethidium bromide.

Statistical analysis

Statistical analysis was performed with the package *SNPassoc* using the *Rstudio* software. Package *SNPassoc* is developed to perform complex genetic analyses for the association studies [19]. The package allows to perform a single SNP analysis in relation to the quantitative or qualitative features. To detect the statistical significance of a SNP under the analysis, likelihood ratio test or LRT is used. The LRT analysis generates an output that describes a sample size with the genotype data and with the effect size. Effect sizes are described differently for the quantitative or qualitative features, In addition, the goodness of the models is described by using the Akaike Information Criterion (AIC) [19].

We started our analysis by analysing the effects of uVNTR and dVNTR separately on all the behaviours characterising the smoking and nicotine addiction. Later we stratified the subjects based on uVNTR or dVNTR to identify genetic interaction between VNTRs. We

analysed FTND as a complex measure and each of its question separately to characterise different aspects of the smoking behaviour and nicotine dependence.

Results

uVNTR polymorphism

uVNTR was polymorphic in our cohort with four different variants: 2 ,3, 4 and 5-repeats. 3-repeat was the commonest and identified in 922 subjects (54 %). The 4-repeat was the second commonest with 765 subjects (44.5 %). 5-repeat variant was detectable in 19 cases (1 %) and 2-repeat variant in 8 cases (0.5 %). As the 5- and 2- repeats were very rare and they are low expression variants as 3-repeat variant, we combined 2 and 5 repeats with the 3-repeats for further analyses. This combination is necessary as the variants with similar functional impact were combined and this way, we generated biallelic variants that are most commonly used by genetic association studies.

The variation in the uVNTR was associated with the question F6 of the Fagerström Test for Nicotine Dependence, "Do you smoke even if you are sick in bed most of the day". Persons with the 3-repeat allele had OR 1.4 (CI 1.1-1.8, P = 0.005) to smoke when sick in bed. Therefore, variant with the low MAOA expression increased the risk for to be more addicted for smoking. No other parts of the smoking behaviour were associated with the variations in the uVNTR.

dVNTR polymorphism

The other VNTR in our study, dVNTR, was also polymorphic in our cohort with 8, 9, 10 and 11 repeats as variants. The most common variant was 9-repeat variant with 974 individuals (55.3 %). The next commonest was 10-repeat variant that was found on 778 subjects (44.2 %). The 11-repeat was identified on 6 subjects (0.3 %) and 8-repeat on 4 subjects (0.2 %). As the 8 and 11 are high expressing variants like the 9-repeat variant, 8 and 11-repeats were combined with 9-repeat variants for further analyses. Again, this combination was justified by the existing functional impact of the variants. The association analysis with dVNTR variation did not reveal any statistically significant associations with the smoking behaviour and nicotine dependence.

Stratification of the uVNTR and dVNTR polymorphisms

Stratification analysis should indicate any genetic interaction between two variants that are functionally meaningful. As previous studies indicated the interaction between dVNTR and uVNTR (ref) we decided to look for associations within the subgroups of the cohort stratified by the uVNTR or by the dVNTR. The most common combinations in our

cohort were 9-3 (51.3%) and 10-4 (40.1%) as predominating haplotypes. Combinations 9-4 (4.5%) and 10-3 (4.1%) were rare in the Vietnamese cohort.

After stratification for the dVNTR genotypes, variations in the uVNTR genotypes became significant for several smoking-related phenotypes (Tables 2 and 3). Subjects with the genotype 10 and 4 have significantly shorter duration of smoking (25 years, Table 2) by years compared to subjects with 10 and 3 repeats (32 years). Similarly, subject with combination between 9 and 4 had longer smoking duration than subjects with 9 and 3. Similarly, subjects with 10 and 3 repeats had significantly higher average FTND score (4.8) compared to persons with any other combination. Interestingly, persons with 10 and 3 repeats had significantly later smoking initiation (20.3 years) compared to other combinations (18 years). On the other hand, persons with 10 and 3 repeats smoked the first cigarette in the morning significantly earlier than subjects with other combinations. Subjects with 10 and 3 repeats made also significantly fewer attempts to quit (Table 2). Almost all significant finding in this part were caused by the combination of two variants that cause low expression (10 and 3) of the MAOA gene and this indicates functional consequences behind these combinations. Similarly, three qualitative measures of the questionnaire were significant after stratification (Table 3). Namely, subjects with 9 and 4 repeats (high-high combination) had significantly higher OR to become a smoker. But again, subjects with 10 and 3 repeats felt more difficult to refrain from smoking when it is prohibited, and they also smoked even they were sick in the bed (Table 3).

Stratification of the study cohort by the uVNTR genotypes confirmed almost all significant associations we identified by the dVNTR stratification (Table 4). Namely, the duration of smoking was longer in persons with combinations of the 3 and 10 or 4 and 9 (Table 4). FTND score was significantly higher in subjects with 3 and 10 repeats. On the other hand, persons with 3 and 10 repeats, started smoking at later age (in average 20 years). Subjects with 3 and 10 combination smoked first cigarette of the day earlier than other combinations and they made significantly less quit attempts (Table 4).

Taken together, our stratification analysis identified significantly higher nicotine dependence and significantly higher smoking persistence in the persons with the uVNTR 3 repeat and dVNTR 10 repeat combination. Both these repeats predict low expression of the MAOA gene and therefore this combination is the lowest expression version compared to other variants.

Discussion

In the present study we analysed two functional VNTRs, likely to affect MAOA expression in a tissue specific or stimulus inducible manner, in the MAOA gene and their impact on the nicotine dependence and smoking behaviour. The MAOA gene has been recognised to regulate impulse control and found to be involved in the substance abuse [20, 21]. The MAOA uVNTR is involved in the gene x environment effect for development of antisocial personality disorder [11]. Smokers have significantly more antisocial characteristics in their behaviour [22]. Combination of the behavioural disorders and potential for substance abuse, makes MAOA gene a good candidate to study in relation of nicotine dependence.

The cohort consisted of 1,804 male subjects of which 1,453 were smokers and 369 non-smokers. FTND was used to measure the nicotine dependence and two VNTRs in the MAOA gene were genotyped to analyse the functional variations in the MAOA gene. FTND is a widely accepted method to evaluate nicotine dependence and has been used for several decades. Likewise, the VNTRs are recognised as common polymorphisms that are responsible for the large part of the genomic variation [23]. In our previous study, we identified the interaction between two VNTRs at the SLC6A4 locus in regulating nicotine dependence [16]. This interaction reflects the functional reciprocal regulation of transcriptional activity between these VNTRs [16]. Based on these existing prerequistes, we decided to test similar interaction between two VNTRs in another genetic locus widely known in behavioural genetics, MAOA.

Smoking itself is a major public health concern as it is a single major cause for premature deaths. It has been shown that smoking will reduce the life expectancy about 10 years [24]. Moreover, long-term smoking causes many diseases that could not develop otherwise or would be rare in the general population [24]. It is clear that reducing prevalence of smoking and helping smokers to quit this detrimental habit would be the easiest solution to improve the quality of life of population. However, quitting of smoking is not easy and it has been estimated that around 30% of smokers have severe nicotine dependence [17]. Improved knowledge of the genetic and behavioural determinants would help to develop personalised support for smoking cessation.

The MAOA gene has been implicated in the genetic susceptibility for a number of psychiatric disorders e.g. mood and panic disorders, impulse control and in the substance abuse [5, 8, 25]. The MAOA enzyme has a central activity in the biochemistry of monoamines and it is involved in the dopamine, serotonin and norepinephrine, all monoamines with fundamental impact on the regulation of behaviour and personality [9, 26]. MAOA activity is variable between individuals and this variability is dependent in part on the transcription of the MAOA gene [7]. Moreover, the genetic polymorphisms (uVNTR) can predict the level of metabolites in the CSF and this correlates with the impulsivity scores or delayed response to the reward [27]. Similarly, delay discounting was strongly associated with the FTND scores and nicotine dependence [28]. This behavioural measure is considered to reflect poor impulse control and it is connected with the difficulties in quitting smoking. MAOA polymorphisms are associated with the attention-deficit/hyperactivity disorder (ADHD), reflecting the involvement of the variations in monoamine neurotransmission in the impulse control [29, 30]. One larger study identified uVNTR to be correlated with the emotional stability and depression [31]. This association was extended to the state anxiety and impulsivity [31]. The authors concluded that the uVNTR in MAOA gene regulates neuroticism and can be treated as a common factor for aggressive behaviours or personality disorders [31]. On the other hand, emotional instability and neuroticism is closely related to the smoking behaviour [32]. Finally, impulsivity disorder symptoms predict increased lifetime risk for smoking and ADHD symptoms interact with genetic variations in predicting the smoking risk [30]. Therefore, behavioural disorders are closely linked to the smoking and nicotine dependence justifying the need to analyse similar genetic markers.

Existing literature supports the idea that variations in the MAOA activity and polymorphisms in its genetic structure are associated with the smoking behaviour [33]. More precisely, persons with certain genetic variants have higher risk for the smoking initiation and persistence [34]. Most of the genetic studies have analysed only the uVNTR in the MAOA gene without any attention to the variations in the dVNTR. The main reason is that the dVNTR has not been addressed in smoking is that only a few studies have addressed this polymorphism and its role in the regulation of MAOA in general. This may be due to the difficulty in PCR of such a rich GC repeat which required significant optimisation in previous paper when analysed [6]. Several fundamental rules have been

described in our studies for the dVNTR. Firstly the, dVNTR has 8, 9, 10 and 11 repeats of the 10-bp motif and the most common variants are 9 and 10 repeats [8]. The other aspect is that 9 -repeat is the most active in transcriptional regulation and is more than 10-times more active than 10-repeat in cell line modes. As a result, the 9-repeat was identified clearly as a high expression variant [8]. In the same paper much stronger transcriptional influence of dVNTR compared to uVNTR was described. This finding was further confirmed in more detailed functional report where differential regulation of uVNTR and dVNTR on alternative isoforms of MAOA was identified [6]. Moreover, the dominance of the dVNTR over the effects of uVNTR was hypothesised. Both of the mentioned papers identified clear haplotype structure between these two VNTRs, with the 9-4 and 10-3 as the most dominant versions of the haplotypes in the Caucasian cohorts.

In our study we identified the most common combinations of the uVNTR and dVNTR haplotypes were 9-3 and 10-4, that is different from Caucasian populations which is 9-4 and 10-3 [6]. This finding can be explained by the population differences and as such is an example to illustrate the importance of the population diversity in genetic association studies. Despite the differences in the haplotype structure, we identified clearly increased nicotine dependence and persistence of smoking behaviour in subjects with the 10-3 combination. This combination predicts low expression of the MAOA and low activity of MAOA enzyme. This finding fits very well with the existing literature, where the low expression genotypes of MAOA were predictive for increased anti-social behaviour [11, 35]. This indicates that even the basic haplotype structures are variable in different populations, the molecular effects and hence the biological outcomes are comparable. Based on our study and previously reported evidence, we can conclude that low MAOA activity genotype is related to smoking and this effect is valid over different populations.

The main limitation of present study is the lack of data for MAOB enzyme and lack of functional analyses. That could give us more comprehensive approach, but MAOB analysis was out of the scope of the present study. Moreover, in case of MAOA we had the information that two VNTR are interacting to regulate its activity, but in case of MAOB we still do not have this type of molecular data. Although the proximity of both genes to one another could suggest that there may be some areas of coordinated expression.

Another limitation of the study is the lack of any nicotine metabolism measurements and all the analysis is based on the questionnaire. Nicotine metabolism could give more reliable smoking information but doing it on the large scale is also challenging, Therefore, getting a larger samples size was favoured over the metabolism analysis and we decided only to use questionnaire approach.

Taken together we identified that in Asian population the MAOA VNTR genetic haplotypes have different distribution, but even then, we were able to identify significant effect of the MAOA low expression haplotype to smoking behaviour and nicotine dependence. In conclusion, low MAOA activity is highly significant risk factor for smoking in different populations and therefore needs more attention for the genetic studies involving multiple populations.

Authors' Contributions

All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript. GK and SK designed the study; GK, EP, conducted the experiments; GK, HDTT, NBTN, LNNH, HMTT, DHX, BHD helped with cohort collection and questionnaires; GK, SK, VJB and JPQ wrote the manuscript, VJB and JPQ provided methodology for genotyping and advised the targets.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Cawthon RM, Pintar JE, Haseltine FP, Breakefield XO. Differences in the structure of A and B forms of human monoamine oxidase. *Journal of neurochemistry* 1981;**37**:363-72

2. Grimsby J, Chen K, Wang LJ, Lan NC, Shih JC. Human monoamine oxidase A and B genes exhibit identical exon-intron organization. *Proceedings of the National Academy of Sciences of the United States of America* 1991;**88**:3637-41

3. Lan NC, Heinzmann C, Gal A, Klisak I, Orth U, Lai E, Grimsby J, Sparkes RS, Mohandas T, Shih JC. Human monoamine oxidase A and B genes map to Xp 11.23 and are deleted in a patient with Norrie disease. *Genomics* 1989;**4**:552-9

4. Bach AW, Lan NC, Johnson DL, Abell CW, Bembenek ME, Kwan SW, Seeburg PH, Shih JC. cDNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymatic properties. *Proceedings of the National Academy of Sciences of the United States of America* 1988;**85**:4934-8

5. Philibert RA, Gunter TD, Beach SR, Brody GH, Madan A. MAOA methylation is associated with nicotine and alcohol dependence in women. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics* 2008;**147B**

6. Manca M, Pessoa V, Lopez AI, Harrison PT, Miyajima F, Sharp H, Pickles A, Hill J, Murgatroyd C, Bubb VJ, Quinn JP. The Regulation of Monoamine Oxidase A Gene Expression by Distinct Variable Number Tandem Repeats. *J Mol Neurosci* 2018;**64**:459-70

7. Sabol SZ, Hu S, Hamer D. A functional polymorphism in the monoamine oxidase a gene promoter. *Human genetics* 1998;**103**

8. Philibert RA, Wernett P, Plume J, Packer H, Brody GH, Beach SR. Gene environment interactions with a novel variable monoamine oxidase A transcriptional enhancer are associated with antisocial personality disorder. *Biological psychology* 2011;**87**

9. Manuck SB, Flory JD, Ferrell RE, Mann JJ, Muldoon MF. A regulatory polymorphism of the monoamine oxidase-A gene may be associated with variability in aggression, impulsivity, and central nervous system serotonergic responsivity. *Psychiatry research* 2000;**95**:9-23

10. Lawson DC, Turic D, Langley K, Pay HM, Govan CF, Norton N, Hamshere ML, Owen MJ, O'Donovan MC, Thapar A. Association analysis of monoamine oxidase A and attention deficit hyperactivity disorder. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics* 2003;**116b**:84-9

11. Caspi A, McClay J, Moffitt TE, Mill J, Martin J, Craig IW, Taylor A, Poulton R. Role of genotype in the cycle of violence in maltreated children. *Science (New York, NY)* 2002;**297**

12. Jha P. Avoidable global cancer deaths and total deaths from smoking. *Nature reviews Cancer* 2009;**9**:655-64

13. Maes HH, Sullivan PF, Bulik CM, Neale MC, Prescott CA, Eaves LJ, Kendler KS. A twin study of genetic and environmental influences on tobacco initiation, regular tobacco use and nicotine dependence. *Psychological medicine* 2004;**34**:1251-61

14. Hohmann S, Zohsel K, Buchmann AF, Blomeyer D, Holz N, Boecker-Schlier R, Jennen-Steinmetz C, Rietschel M, Witt SH, Schmidt MH, Esser G, Meyer-Lindenberg A, Banaschewski T, Brandeis D, Hohm E, Laucht M. Interacting effect of MAOA genotype and maternal prenatal smoking on aggressive behavior in young adulthood. *Journal of neural transmission (Vienna, Austria : 1996)* 2016;**123**:885-94

15. Kim SW, Lee JM, Ban WH, Park CK, Yoon HK, Lee SH. Smoking habits and nicotine dependence of North Korean male defectors. *The Korean journal of internal medicine* 2016;**31**:685-93

16. Koks G, Prans E, Tran HDT, Ngo NBT, Hoang LNN, Tran HMT, Cao Ngoc T, Doan Phuoc T, Ho XD, Ho Duy B, Lattekivi F, Quinn J, Koks S. Genetic Interaction Between Two VNTRs in the SLC6A4 Gene Regulates Nicotine Dependence in Vietnamese Men. *Front Pharmacol* 2018;**9**:1398

17. Koks G, Tran HDT, Ngo NBT, Hoang LNN, Tran HMT, Ngoc TC, Phuoc TD, Dung Ho X, Duy BH, Lattekivi F, Koks S. Cross-Sectional Study to Characterise Nicotine Dependence in Central Vietnamese Men. *Subst Abuse* 2019;**13**:1178221818822979

18. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *Journal of biomedical informatics* 2009;**42**:377-81

19. Gonzalez JR, Armengol L, Sole X, Guino E, Mercader JM, Estivill X, Moreno V. SNPassoc: an R package to perform whole genome association studies. *Bioinformatics* 2007;**23**:644-5

20. Contini V, Marques FZ, Garcia CE, Hutz MH, Bau CH. MAOA-uVNTR polymorphism in a Brazilian sample: further support for the association with impulsive behaviors and alcohol dependence. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics* 2006;**141b**:305-8

21. Tiili EM, Mitiushkina NV, Sukhovskaya OA, Imyanitov EN, Hirvonen AP. The genotypes and methylation of MAO genes as factors behind smoking behavior. *Pharmacogenetics and genomics* 2017;**27**:394-401

22. Patton D, Barnes GE, Murray RP. A personality typology of smokers. *Addictive behaviors* 1997;**22**:269-73

23. Lilleoja R, Sarapik A, Reimann E, Reemann P, Jaakma U, Vasar E, Koks S. Sequencing and annotated analysis of an Estonian human genome. *Gene* 2012;**493**:69-76

24. Koks G, Fischer K, Koks S. Smoking-related general and cause-specific mortality in Estonia. *BMC public health* 2017;**18**:34

25. Howe AS, Buttenschon HN, Bani-Fatemi A, Maron E, Otowa T, Erhardt A, Binder EB, Gregersen NO, Mors O, Woldbye DP, Domschke K, Reif A, Shlik J, Koks S, Kawamura Y, Miyashita A, Kuwano R, Tokunaga K, Tanii H, Smoller JW, Sasaki T, Koszycki D, De Luca V. Candidate genes in panic disorder: meta-analyses of 23 common variants in major anxiogenic pathways. *Molecular psychiatry* 2016;**21**:665-79

26. Fergusson DM, Boden JM, Horwood LJ, Miller A, Kennedy MA. Moderating role of the MAOA genotype in antisocial behaviour. *Br J Psychiatry* 2012;**200**:116-23

27. He Y, Hogrefe CE, Grapov D, Palazoglu M, Fiehn O, Turck CW, Golub MS. Identifying individual differences of fluoxetine response in juvenile rhesus monkeys by metabolite profiling. *Translational psychiatry* 2014;**4**:e478

28. Sweitzer MM, Donny EC, Dierker LC, Flory JD, Manuck SB. Delay discounting and smoking: association with the Fagerstrom Test for Nicotine Dependence but not cigarettes smoked per day. *Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco* 2008;**10**:1571-5

29. Bidwell LC, Garrett ME, McClernon FJ, Fuemmeler BF, Williams RB, Ashley-Koch AE, Kollins SH. A preliminary analysis of interactions between genotype, retrospective ADHD symptoms, and initial reactions to smoking in a sample of young adults. *Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco* 2012;**14**:229-33

30. McClernon FJ, Fuemmeler BF, Kollins SH, Kail ME, Ashley-Koch AE. Interactions between genotype and retrospective ADHD symptoms predict lifetime smoking risk in a sample of young adults. *Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco* 2008;**10**:117-27

31. Rodriguez-Ramos A, Moriana JA, Garcia-Torres F, Ruiz-Rubio M. Emotional stability is associated with the MAOA promoter uVNTR polymorphism in women. *Brain Behav* 2019;**9**:e01376

32. O'Gara C, Knight J, Stapleton J, Luty J, Neale B, Nash M, Heuzo-Diaz P, Hoda F, Cohen S, Sutherland G, Collier D, Sham P, Ball D, McGuffin P, Craig I. Association of the serotonin transporter gene, neuroticism and smoking behaviours. *Journal of human genetics* 2008;**53**:239-46

33. Berlin I, Anthenelli RM. Monoamine oxidases and tobacco smoking. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum* 2001;**4**:33-42

34. Munafo M, Clark T, Johnstone E, Murphy M, Walton R. The genetic basis for smoking behavior: a systematic review and meta-analysis. *Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco* 2004;**6**:583-97

35. Kim-Cohen J, Caspi A, Taylor A, Williams B, Newcombe R, Craig IW, Moffitt TE. MAOA, maltreatment, and gene-environment interaction predicting children's mental health: new evidence and a meta-analysis. *Molecular psychiatry* 2006;**11**:903-13